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ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

EXAMINER	
DUNSTON, JENNIFER ANN	
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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte EDWARD REBAR and
ANDREW JAMIESON

Appeal 2010-006590
Application 10/055,711
Technology Center 1600

Before DEMETRA J. MILLS, ERIC GRIMES, and LORA M. GREEN, *Administrative Patent Judges*.

GRIMES, *Administrative Patent Judge*.

DECISION ON APPEAL[1]

This is an appeal under 35 U.S.C. § 134 involving claims to a polynucleotide encoding a non-naturally-occurring zinc-finger binding protein. The Examiner has rejected the claims as obvious. We have

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[1] The two-month time period for filing an appeal or commencing a civil action, as recited in 37 C.F.R. § 1.304, or for filing a request for rehearing, as recited in 37 C.F.R. § 41.52, begins to run from the "MAIL DATE" (paper delivery mode) or the "NOTIFICATION DATE" (electronic delivery mode) shown on the PTOL-90A cover letter attached to this decision.

jurisdiction under 35 U.S.C. § 6(b). We affirm.

STATEMENT OF THE CASE

The Specification discloses that a "'canonical' zinc finger refers to a zinc-coordinating component *(e.g.,* zinc finger) of a zinc finger protein having the general amino acid sequence: X_3-Cys-X_{2-4}-Cys-X_{12}-His-X_{1-7}-His-X_4 (SEQ ID NO. 2) where X is any amino acid (also known as a C2H2 zinc finger)" (Spec. 10: 14-17, as amended August 19, 2005). Zinc fingers that differ from the C2H2 pattern are known as "non-canonical" zinc fingers (see *id.* at 7: 6-11).

Claims 25-28, 30-32, 36, 37, 39-41, and 53-57 are on appeal. Claim 30 is representative and reads as follows:

30. An isolated polynucleotide encoding a non-naturally-occurring zinc-finger binding protein comprising a non-canonical zinc finger component, wherein:

(i) said non-canonical zinc finger component contains a beta turn comprising two amino-terminal zinc coordinating cysteine or histidine residues and an alpha helix comprising two carboxy-terminal zinc coordinating cysteine or histidine residues, wherein at least one of the zinc coordinating residues is a histidine residue and at least one of the zinc coordinating residues is a cysteine residue;

(ii) the non-canonical zinc finger component comprises 1, 2, 3, 4, 6 or 7 amino acids between the two carboxy-terminal zinc coordinating residues and 2, 3 or 4 amino acids between the two amino-terminal zinc coordinating residues; and

(iii) the non-canonical zinc-finger binding domain protein comprises a recognition helix of at least 7 amino acids in length, wherein the recognition helix is nonnaturally occurring and is engineered to bind to a target nucleic acid sequence in a plant cell.

The claims stand rejected under 35 U.S.C. § 103(a) as follows:

- Claims 25-28, 30-32, 36, 37, 39-41, and 53-57 in view of Barbas '201[2] and Filippova;[3]
- Claims 25-28, 30-32, 36, 39-41, and 53-57 in view of Barbas '728[4] and Filippova; and
- Claim 37 in view of Barbas '728, Filippova, and Guyer.[5]

I.

Issue

The Examiner has rejected claims 25-28, 30-32, 36, 37, 39-41, and 53-57 as being obvious in view of Barbas '201 and Filippova. The claims have not been argued separately and therefore stand or fall together. 37 C.F.R. § 41.37(c)(1)(vii).

The Examiner finds that Barbas '201 discloses "nucleic acid molecules encoding zinc finger proteins that bind to a target nucleotide sequence" (Answer 4). The Examiner finds that Barbas '201 discloses that the binding specificity of zinc finger proteins is determined based on a seven-amino-acid recognition helix, and "any naturally occurring zinc finger protein can be used as a framework (or backbone) to derive a non-naturally occurring zinc finger with DNA binding specificity determined by

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[2] Barbas, III et al., US 7,151,201, Dec. 19, 2006
[3] Galina N. Filippova et al., *An Exceptionally Conserved Transcriptional Repressor, CTCF, Employs Different Combinations of Zinc Fingers to Bind Diverged Promoter Sequences of Avian and Mammalian c-myc Oncogenes*, 16 MOLECULAR AND CELLULAR BIOLOGY, 2802-2813 (1996)
[4] Barbas, III et al., US 7,329,728, Feb. 12, 2008
[5] Dave Guyer et al., *Activation of Latent Transgenes in Arabidopsis Using a Hybrid Transcription Factor,* 149 GENETICS, 633-639 (1998)

alterations in the alpha helix using known design rules" (*id.* at 5). The Examiner further finds that Barbas '201 discloses that "the target nucleotide sequence can be present in a plant cell and can be a promoter sequence" (*id.*). The Examiner finds that Filippova discloses "nucleic acid molecules encoding the 11 zinc fingers of CTCF protein" (*id.* at 6), and that "[f]inger 11 of the CTCF DNA-binding protein contains the amino acid sequence CSKCGKTFTRRNTMARHADNC" (*id.*), which is a non-canonical zinc finger (*id.*).

The Examiner concludes that it

> would have been obvious to one or ordinary skill in the art … to include the framework sequence encoding [Filippova's] finger 11 (i.e., the CCHC zinc finger) of the CTCF protein … in the nucleic acid molecules of Barbas ['201] …, where the finger 11 sequence has been … engineered to bind … a target nucleic acid sequence taught by Barbas ['201] … to achieve the predictable result of making a polynucleotide that encodes a zinc finger polypeptide that binds to a plant promoter sequence.

(*Id.* at 6-7.)

Appellants contend that the cited references would not have suggested altering the recognition helix of the eleventh finger of Filippova's CTCF protein so that it would bind to a target in a plant gene (Appeal Br. 8-12). Appellants also contend that Barbas '201 does not suggest or enable combining a zinc finger domain from one protein with zinc fingers from an different protein (Reply Br. 7-12).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that one of ordinary skill in the art would have considered it obvious to combine the non-canonical zinc finger domain of Filippova's zinc-finger protein with the non-naturally occurring zinc finger proteins taught by Barbas '201?

Findings of Fact

1. Barbas '201 discloses "the use of zinc finger proteins and fusions of said proteins to regulate gene expression and metabolic pathways in plants" (Barbas '201, abstract).

2. Barbas '201 discloses that "[a]ny types of zinc finger protein can be used in the present method. But preferably, … a framework from a plant zinc finger protein can be used. Alternatively, synthetic zinc finger proteins or non-naturally-occurring zinc finger proteins can be used" (*id.* at col. 5, ll. 6-11).

3. Barbas '201 discloses that "[e]ach finger usually comprises an approximately 30 amino acid[], zinc-chelating, DNA-binding subdomain" (*id.* at col. 10, ll. 65-67).

4. Barbas '201 discloses that the term "'framework (or backbone) derived from a naturally occurring zinc finger protein' means that the protein or peptide sequence … involved in non-sequence specific binding with a target nucleotide sequence is not substantially changed from its natural sequence" (*id.* at col. 11, ll. 14-19).

5. Barbas '201 discloses that its zinc finger protein can comprise wild-type zinc fingers that bind desired nucleic acid sequences or zinc fingers that have been mutated to bind to a desired nucleic acid sequence (*id.* at col. 18, l. 65 to col. 19, l. 34).

6. Barbas '201 discloses that the "zinc finger polypeptides used in the present method can be engineered to recognize a selected target site in the gene of choice…. A zinc finger polypeptide can be designed or selected to bind to any suitable target site in the target gene, with high affinity." (*Id.* at col. 20, ll. 3-14.)

7. Barbas '201 discloses that "[z]inc finger proteins useful in the method can be made by any recombinant DNA technology method for gene construction…. Preferred for cost and flexibility is the use of PCR primers that encode a finger sequence or part thereof with known base pair specificity, and that can be reused or recombined to create new combinations of fingers and ZFP sequences" (*id.* at col. 20, ll. 30-40).

8. Filippova discloses that cDNA encoding a human transcription factor (CTCF) that includes eleven zinc fingers (Filippova 2802, right col.).

9. Filippova discloses that the amino acid sequence of finger 11 of human CTCF is CSKCGKTFTRRNTMARHADNC (*id.* at 2805, Fig. 2A).

10. Finger 11 of human CTCF is a non-canonical zinc finger (see Spec. 7: 6-11)

11. Filippova discloses that "fingers 3 to 11 mediate CTCF binding to the human [c-myc] promoter" (Filippova 2802, abstract).

Analysis

Claim 30 is directed to an isolated polynucleotide encoding a non-naturally-occurring zinc-finger protein comprising, among other things, a non-canonical zinc finger having an amino-terminal beta turn and a carboxy-terminal alpha helix that each comprise two cysteine or histidine residues (at least one of each in the zinc finger) with specified spacing.

Barbas '201 discloses polynucleotides encoding a non-naturally-occurring zinc-finger protein that is engineered to bind to a target nucleic acid sequence in a plant cell (FFs 1, 2). Barbas '201 discloses that one preferred method of making its zinc finger protein uses PCR primers to amplify DNA encoding a finger sequence with known DNA-binding specificity that can be recombined to create new combinations of zinc

fingers (FF 7). Filippova discloses a human protein that comprises a non-canonical zinc finger that meets the structural requirements specified in claim 30 and that helps mediate sequence-specific binding of the zinc finger protein to DNA. In view of these disclosures, it would have been obvious to one of skill in the art to use DNA encoding the non-canonical zinc finger disclosed by Filippova in the zinc finger-encoding nucleic acid molecule of Barbas '201 in order to confer on the recombinant protein of Barbas '201 the DNA-binding specificity of Filippova's zinc finger.

The arguments presented by Appellants in the Appeal Brief are addressed to whether it would have been obvious to alter the noncanonical zinc finger in Filippova's CTCF protein with an expectation of obtaining a zinc finger protein that binds to a target site in a plant cell. See, e.g., Appeal Br. 8 ("Specifically, when using Filippova's CTCF as the framework (backbone), the skilled artisan must have been taught by Barbas '201 that altering the recognition helix of the only C3H finger (the 11th finger) of CTCF would result in a protein in which this altered C3H finger bound to a target site in a plant gene."); *id.* at 12 ("In sum, the references do not teach or suggest altering the recognition helix of Filippova's 11th finger of CTCF so as to bind to a target site in a plant gene.").

These arguments, however, are not directed to the rationale on which the Examiner relies. Although the Examiner also addresses modifying the finger 11 sequence to bind Barbas '201's target sequence (Answer 6-7), the rejection is based on the obviousness of "includ[ing] the framework sequence encoding finger 11 (i.e., the CCHC zinc finger) of the CTCF protein of Filippova et al in the nucleic acid molecules of Barbas" (Answer

6). We agree with the Examiner's conclusion that including Filippova's finger 11 in Barbas '201's recombinant protein would have been obvious.

Appellants also argue that "Barbas '201 does not teach or suggest isolating a single zinc finger from its natural context and then combining the individual finger with different fingers from other proteins" (Reply Br. 7). Appellants argue that "Barbas '201 refers only to one multi-finger protein framework that is then modified in its recognition helix region" (*id.* at 10).

This argument is not persuasive because, as discussed above, Barbas '201 discloses that a preferred method of making its zinc finger proteins is "the use of PCR primers that encode a finger sequence … with known base pair specificity, and that can be … recombined to create new combinations of fingers" (FF 7). Thus, Barbas '201 expressly suggests combining DNA encoding a known zinc finger with different zinc fingers to create new combinations.

Appellants also argue that "[t]here is no disclosure whatsoever in Barbas '201 regarding how to combine a single finger from one framework with finger(s) from another framework. As such, the skilled artisan would have no expectation that zinc finger proteins combining Filippova's 11^{th} finger with Barbas '201's frameworks (e.g., Sp1C) would be functional." (Reply Br. 11.)

This argument is also unpersuasive. Barbas '201 discloses that known PCR techniques can be used to amplify DNA encoding zinc finger sequences with known specificity and combine them with DNA encoding other zinc fingers to create new combinations (FF 7). A patent is presumed to enable what it discloses, *see Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1355 (Fed. Cir. 2003) ("[A] presumption arises that both the

claimed and unclaimed disclosures in a prior art patent are enabled."), and the applicant bears the burden of proving that it does not, *see id.* ("The applicant, however, can then overcome that rejection by proving that the relevant disclosures of the prior art patent are not enabled."). Appellants have not provided adequate evidence to overcome the presumption that Barbas '201 enables those skilled in the art to combine zinc fingers from different zinc finger proteins to create new combinations of zinc fingers.[6]

Conclusion of Law

The evidence of record supports the Examiner's conclusion that one of ordinary skill in the art would have considered it obvious to combine the non-canonical zinc finger domain of Filippova's zinc-finger binding protein with the non-naturally occurring zinc finger proteins taught by Barbas '201.

II.

Issue

The Examiner has rejected claims 25-28, 30-32, 36, 39-41 and 53-57 as being obvious in view of Barbas '728 and Filippova. The claims have not been argued separately and therefore stand or fall with claim 30. 37 C.F.R. § 41.37(c)(1)(vii).

The Examiner finds that Barbas '728 discloses a nucleic acid encoding a fusion protein that includes DNA-binding, ligand-binding, and transcription-modulating domains (Answer 8). The Examiner also finds that

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[6] Appellants also cite Barbas '728 as evidence that "the skilled artisan believed that framework residues could impact binding and, as such, only when the recognition helix of certain Cys2His2 frameworks were altered was binding function in any way predictable" (Appeal Br. 8-9). This argument is addressed below.

Barbas '728 discloses that "the DNA binding domain includes at least three zinc finger modular units" and that "the ability to construct zinc fingers with unique specificity, … permits targeting and ligand-dependent control of expression of specific … genes" (*id.*). The Examiner further finds that Barbas '728 discloses that "rules for creating synthetic zinc fingers with specificity to any desired target sequence are known" (*id.*). The Examiner also finds that Barbas '728 discloses that "any framework sequences known in the art to function as part of a zinc finger protein" can be used (*id.*).

The Examiner relies on Filippova as discussed previously. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to include Filippova's "framework sequence encoding finger 11 (i.e., the CCHC zinc finger) … where the finger 11 sequence has been modified to include a recognition helix that … bind[s] the … target nucleic acid sequence taught by Barbas … to achieve the predictable result of making a polynucleotide that encodes a zinc finger polypeptide that binds to a plant promoter sequence" (*id.* at 10).

Appellants contend that "Barbas '728 teaches away from using Filippova's CTCF framework by … teaching that it is entirely unpredictability [sic] that altering the recognition helix of frameworks other than canonical Zif268 or Spl" would produce a protein with the desired binding characteristics (Appeal Br. 12) and that Barbas '728 teaches that a zinc finger's binding affinity is unpredictable when it is isolated from its natural framework (Reply Br. 18).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that the cited references would

have made it obvious to use Filippova's non-canonical zinc finger in the fusion proteins suggested by Barbas '728?

Additional Findings of Fact

12. Barbas '728 discloses "[f]usion proteins for use as ligand-dependent transcriptional regulators" (Barbas '728, abstract).

13. Barbas '728 discloses that the "fusion proteins include a nucleotide binding domain operatively linked to a ligand-binding domain. They also can include a transcription regulating domain" (*id.*).

14. Barbas '728 discloses that the "nucleotide binding domain is a zinc-finger peptide that binds to a targeted contiguous nucleotide sequence of from 3 to about 18 nucleotides" (*id.*).

15. Barbas '728 discloses that the "fusion proteins can be used in plant species as well as animals. Transgenic plants resistant to particular bacterial or viral pathogens can be produced." (*Id.* at col. 3, ll. 12-14.)

16. Barbas '728 discloses that a "zinc finger-nucleotide binding peptide domain contains a unique heptamer (contiguous sequence of 7 amino acid residues) … [that] determines binding specificity to a target nucleotide" (*id.* at col. 20, ll. 43-47).

17. Barbas '728 discloses that the nucleic acid binding domain, or DBD, "includes at least one zinc finger modular unit and is engineered to bind to targeted genes…. Any zinc finger or modular portions thereof can be used. The DBD replaces or supplements the naturally-occurring zinc finger domain in the receptor from which the ligand binding domain is derived." (*Id.* at col. 3, ll. 51-58.)

18. Barbas '728 discloses that "[m]odular zinc protein units can be combined so that the resulting domain specifically binds to any targeted sequence" (*id.* at col. 3, l. 66 to col. 4, l. 1).

19. Barbas '728 discloses that "[n]aturally occurring zinc finger proteins generally contain multiple repeats of the zinc finger motif.... Any such zinc finger or combinations of modular units thereof is intended for use herein." (*Id.* at col. 20, ll. 28-42.)

20. Barbas '728 discloses that a "peptide nucleotide-binding domain can include any β-sheet and framework sequences known in the art to function as part of a zinc finger protein" (*id.* at col. 20, ll. 51-53).

Analysis

Barbas '728 discloses nucleic acids encoding fusion proteins for use as ligand-dependent transcriptional regulators in both plants and animals. Barbas '728 discloses that the fusion proteins include a ligand-binding domain, a transcription-regulating domain, and a nucleotide-binding domain that is a zinc-finger peptide that binds to a targeted nucleotide sequence.

Barbas '728 discloses that zinc fingers can be constructed with unique specificity, and that naturally occurring zinc fingers can be combined to target a desired nucleotide sequence. Filippova discloses a human zinc finger protein that comprises a non-canonical zinc finger that meets the structural requirements specified in claim 30 and that helps mediate binding of the protein to DNA. In view of these disclosures, it would have been obvious to use DNA encoding the non-canonical zinc finger of Filippova in the fusion protein-encoding nucleic acid of Barbas '728 in order to confer on the recombinant protein the DNA-binding specificity of Filippovas's zinc finger.

Appellants argue that Barbas '728 teaches that "altered recognition helices function only in certain frameworks," specifically, canonical Cys2His2 frameworks (Reply Br. 14; see also Appeal Br. 11). Appellants argue that "Barbas '728 clearly teaches that once a finger is isolated from its natural framework and even when it is recombined with other fingers from the same framework, binding affinity is entirely unpredictable" (Reply Br. 18).

This argument is not persuasive. Barbas '728 expressly discloses that its nucleotide-binding domain can include any zinc finger protein β-sheet and framework sequences known in the art (FF 20) and that naturally occurring zinc fingers can be combined to target its fusion protein to a desired nucleotide sequence (FFs 17-19). These disclosures would have provided a skilled worker with a reasonable expectation that Filippova's zinc finger would retain its DNA-binding specificity as part of the fusion protein disclosed by Barbas '728. While Barbas '728 describes an embodiment in which "amino acid positions -2 to 6 of the DNA recognition helices are either grafted into a Zif268 … or an Sp1C framework" (Barbas '728, col. 42, ll. 16-22), its disclosure is not limited to that embodiment. Appellants have not provided adequate evidence to show that Barbas '728 is not enabling for its disclosure of combining naturally occurring zinc fingers in its fusion proteins. *See Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1355 (Fed. Cir. 2003).

Conclusion of Law

The evidence of record supports the Examiner's conclusion that the cited references would have made it obvious to use Filippova's non-canonical zinc finger in the fusion proteins suggested by Barbas '728.

III.

The Examiner has rejected claim 37 under 35 U.S.C. § 103(a) as being obvious in view of Barbas '728, Filippova, and Guyer.

The Examiner finds that Barbas '728 and Filippova suggest the polynucleotide of claim 30, as discussed above, and that Guyer would have made obvious the additional limitations of claim 37 (Answer 11). We agree with the Examiner's reasoning and conclusion.

Appellants do not dispute that Guyer would have suggested the limitations added to claim 30 by claim 37, but contend that the Guyer reference does not cure the deficiencies of Barbas '728 and Filippova in suggesting the invention of claim 30 (Appeal Br. 13).

This argument is not persuasive for the reasons discussed above.

SUMMARY

We affirm the rejection of claims 25-28, 30-32, 36, 37, 39-41, and 53-57 under 35 U.S.C. § 103(a).

TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

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ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO CA 94303